

CLAIMS

1. A method of preparing normalized and/or subtracted cDNAs characterized by comprising the steps of:
 - I) preparing uncloned cDNAs (testers);
 - II) preparing polynucleotides (drivers) for normalization and/or subtraction;
 - III) conducting normalization and/or subtraction and removing tester/driver hybrids and non-hybridized polynucleotide drivers; and
 - IV) recovering the normalized and/or subtracted cDNA.
2. The method of claim 1, wherein the cDNA tester of step I) is reverse transcript of mRNA in form of uncloned cDNA.
3. The method of claim 1 wherein said cDNA tester is single strand.
4. The method of any of claims 1-3 wherein in step III), normalization is conducted first, followed by subtraction.
5. The method of any of claims 1-3 wherein in step III), subtraction is conducted first, followed by normalization.
6. The method of any of claims 1-3 wherein in step III), said tester and normalization and subtraction drivers are mixed together and normalization and subtraction are conducted as a single step.
7. The method of any of claims 1-6 wherein said normalized and/or subtracted cDNA is long-strand, full-coding, and/or full-length cDNA.
8. The method of any of claims 1-7 wherein step III) comprises the addition of an enzyme capable of cleaving single-strand RNA driver nonspecifically bound to single strand cDNA and the cleaved single strand RNA driver is removed.
9. The method of claim 8 wherein said enzyme is single-strand-specific RNA endonuclease.
10. The method of claim 8 wherein said enzyme is either selected from the group consisting of RNase I, RNaseA, RNase4, RNaseT1, RNaseT2, RNase2, and RNase3, or comprises a mixture thereof.

Sub
A2

11. The method of claim 8 wherein said enzyme is RNase I.

12. The method of any of claims 1-11 wherein said cDNA tester is prepared by CAP-trapping the 5' end of RNA.

13. The method of any of claims 1-12 wherein the preparation of said cDNA tester comprises the following steps:

- (1) synthesizing first strand cDNA by means of reverse transcriptase forming mRNA/cDNA hybrids;
- (2) chemically binding a tag molecule to the diol structure of the 5' CAP(⁷MeG_{ppp}N) site of mRNA forming hybrids;
- (3) trapping long-strand, full-coding, and/or full-length cDNA hybrids; and
- (4) removing single strand mRNA through digestion with an enzyme capable of cleaving single strand mRNA.

14. The method of claim 13 wherein said tag molecule is digoxigenin, biotin, avidin, or streptavidin.

15. The method of any of claims 1-14, wherein said polynucleotide driver for normalization and/or subtraction is RNA and/or DNA.

16. The method of claim 15, wherein said DNA driver is cDNA.

17. The method of any of claims 1-16 wherein said normalization driver comprises cellular mRNA from the same library, the same tissue, or the same cDNA population as what is to be normalized.

18. The method of any of claims 1-16 wherein said normalization driver comprises single strand cDNA obtained from the same library, the same tissue, or the same cDNA population as what is to be normalized.

19. The method of any of claims 1-16 wherein said subtraction driver comprises cellular mRNA from a library, tissue, or cDNA population differing from what is to be subtracted.

20. The method of any of claims 1-16 wherein said subtraction driver comprises single strand cDNA from a library, tissue, or cDNA population differing from what is to be normalized.

21. The method of any of claims 1-20, further comprising a step V) of preparing a second strand of recovered cDNA and performing cloning.

22. . A method of preparing normalized and/or subtracted cDNAs characterized by comprising the steps of:

- I) preparing cDNAs (testers) not cloned in plasmid;
- II) preparing polynucleotides (drivers) for normalization and/or subtraction;
- III) conducting normalization and/or subtraction and removing tester/driver hybrids and non-hybridized polynucleotide drivers; and
- IV) recovering the normalized and/or subtracted cDNA.

23. . The method of claim 22, wherein in step III), normalization is conducted first, followed by subtraction.

24. The method of claim 22, wherein in step III), subtraction is conducted first, followed by normalization.

25. The method of claim 22, wherein in step III), said tester and normalization and subtraction drivers are mixed together and normalization and subtraction are conducted as a single step.

26. The method of any of claims 22-25, wherein said normalized and/or subtracted cDNA is long-strand, full-coding, and/or full-length cDNA.

27. The method of any of claims 22-26, wherein step III) comprises the addition of an enzyme capable of cleaving single-strand RNA driver nonspecifically bound to single strand cDNA and the cleaved single strand RNA driver is removed.

28. The method of claim 27, wherein said enzyme is single-strand-specific RNA endonuclease.

29. The method of claim 27, wherein said enzyme is either selected from the group consisting of RNase I, RNaseA, RNase4, RNaseT1, RNaseT2, RNase2, and RNase3, or comprises a mixture thereof.

30. The method of claim 27, wherein said enzyme is RNase I.

Sub
A6

0935592-032404

Sub
A7

31. The method of any of claims 22-30, wherein said cDNA tester is prepared by CAP-trapping the 5' end of RNA.
32. The method of any of claims 22-31, wherein said normalization driver comprises cellular mRNA from the same library, the same tissue, or the same cDNA population as what is to be normalized.
33. The method of any of claims 22-31, wherein said normalization driver comprises single strand cDNA obtained from the same library, the same tissue, or the same cDNA population as what is to be normalized.
34. The method of any of claims 22-31, wherein said subtraction driver comprises cellular mRNA from a library, tissue, or cDNA population differing from what is to be subtracted.
35. The method of any of claims 22-31, wherein said subtraction driver comprises single strand cDNA from a library, tissue, or cDNA population differing from what is to be normalized.
36. The method of any of claims 22-35, further comprising a step V) of preparing a second strand of recovered cDNA and performing cloning.
37. A method of preparing normalized and subtracted cDNA comprising the steps of:
 - I) preparing cDNAs (tester);
 - II) preparing polynucleotides (drivers) for normalization and subtraction;
 - III) conducting the normalization and subtraction as a single step by mixing together the tester and the drivers; and
 - IV) recovering the normalized and subtracted cDNA.
38. The method of claim 37, wherein the cDNA tester is cloned or uncloned cDNA.
39. The method of claim 37, wherein cDNA tester is reverse transcript of mRNA in form of uncloned cDNA.
40. The method of claim 37, wherein the cDNA tester is single strand.
41. The method of any of claims 37-40, wherein said normalized and subtracted cDNA is long-strand, full-coding, and/or full-length cDNA.

Q1
42. The method of any of claims 37-41, wherein step III) comprises the addition of an enzyme capable of cleaving single-strand RNA driver nonspecifically bound to single strand cDNA and the cleaved single strand RNA driver is removed.

43. The method of claim 42, wherein said enzyme is single-strand-specific RNA endonuclease.

44. The method of claim 42, wherein said enzyme is either selected from the group consisting of RNase I, RNaseA, RNase4, RNaseT1, RNaseT2, RNase2, and RNase3, or comprises a mixture thereof.

45. The method of claim 42, wherein said enzyme is RNase I.

46. The method of any of claims 37-45, wherein said cDNA tester is prepared by CAP-trapping 5' end of RNA.

47. The method of any of claims 37-46, wherein the preparation of said cDNA tester comprises the following steps:

- 0993592-08401
T01299-2552660
- (1) synthesizing first strand cDNA by means of reverse transcriptase forming mRNA/cDNA hybrids;
 - (2) chemically binding a tag molecule to the diol structure of the 5' CAP(⁷MeG_{ppp}N) site of mRNA forming hybrids;
 - (3) trapping long-strand, full-coding, and/or full-length cDNA hybrids; and
 - (4) removing single strand mRNA through digestion with an enzyme capable of cleaving single strand mRNA.

48. The method of claim 47, wherein said tag molecule is digoxigenin, biotin, avidin, or streptavidin.

49. The method of any of claims 37-48, wherein said polynucleotide driver for normalization and/or subtraction is RNA and/or DNA.

50. The method of claim 49, wherein said DNA driver is cDNA.

51. The method of any of claims 37-50, wherein said normalization driver comprises cellular mRNA from the same library, the same tissue, or the same cDNA population as what is to be normalized.

52. The method of any of claims 37-50, wherein said normalization driver comprises single strand cDNA obtained from the same library, the same tissue, or the same cDNA population as what is to be normalized.

53. The method of any of claims 37-50, wherein said subtraction driver comprises cellular mRNA from a library, tissue, or cDNA population differing from what is to be subtracted.

54. The method of any of claims 37-50, wherein said subtraction driver comprises single strand cDNA from a library, tissue, or cDNA population differing from what is to be normalized.

55. The method of any of claims 37-54, further comprising a step V) of preparing a second strand of recovered cDNA and performing cloning.

56. A method of preparing normalized and/or subtracted cDNA comprising the steps of:

- (a) preparing cDNA (tester);
- (b) preparing normalization and/or subtraction RNA (driver);
- (c) conducting normalization and/or subtraction in two steps in any order, or conducting normalization/subtraction as a single step and mixing the normalization/subtraction RNA driver with said cDNA tester;
- (d) adding an enzyme capable of cleaving single strand sites on RNA drivers non-specifically bound to cDNA tester;
- (e) removing said single strand RNA driver cleaved in step d) from the tester and removing tester/driver hybrids; and
- (f) recovering the normalized and/or subtracted cDNA.

57. The method of claim 56, wherein the cDNA tester is cloned or uncloned cDNA.

58. The method of claim 56, wherein the cDNA tester is reverse transcript of mRNA in form of uncloned cDNA.

59. The method of claim 56, wherein said cDNA tester is single strand

60. The method of any of claims 56-59, wherein in step c), normalization is conducted first, followed by subtraction.

61. The method of any of claims 56-59, wherein in step c), subtraction is conducted first, followed by normalization.

62. The method of any of claims 56-59, wherein in step c), said tester and normalization and subtraction drivers are mixed together and normalization and subtraction are conducted as a single step.

63. The method of any of claims 56-62, wherein said normalized and/or subtracted cDNA is long-strand, full-coding, and/or full-length cDNA.

64. The method of any of claims 56-65, wherein the enzyme of said step d) is either selected from the group consisting of RNase I, RNaseA, RNase4, RNaseT1, RNaseT2, RNase2, and RNase3, or comprises a mixture thereof.

65. The method of any of claims 56-63, wherein the enzyme of said step d) is RNase I.

66. The method of any of claims 56-65, wherein said cDNA tester is prepared by CAP-trapping the 5' end of RNA.

67. The method of any of claims 56-66, further comprising the step g) of preparing a second strand of recovered cDNA and performing cloning.

68. The method of any of claims 1-67, wherein said tester/driver hybrids are bound to tag molecules.

69. The method of claim 68, wherein said tag molecule is avidin, streptavidin, biotin, digoxigenin, an antibody, or an antigen.

70. The method of any of claims 1-69, wherein said tester/driver hybrids are removed through the use of a matrix.

71. The method of claim 70, wherein said matrix is comprised of magnetic beads or agarose beads.

72. The method of claim 71, wherein said magnetic beads or agarose beads are covered by or bound to any tag molecule capable of binding to tag molecules bound to a tester/driver hybrids.

73. The method of claim 71, wherein said magnetic beads or agarose beads are covered by or bound to a tag molecule capable of binding to avidin, streptavidin, biotin, digoxigenin, an antibody, or an antigen bound to a tester/driver hybrid.

Sub
A13

74. The method of claim 72 or 73, wherein said antibody covering said beads or said antibody binding said beads is an antiantigen antibody, antibiotin antibody, antiavidin antibody, antistreptavidin antibody, or antidigoxigenin antibody.

75. The method of any of claims 1-74, wherein said tester/driver hybrid is removed through the use of streptavidin/phenol.

76. The method of any of claims 1-75, wherein hydroxyapatite and nonlabeled RNA are employed to remove said tester/driver hybrid.

77. A method of removing RNA nonspecifically bound to DNA by processing nonspecifically bound RNA/DNA hybrids with an enzyme capable of degrading single strand RNA.

78. The method of claim 77, wherein said enzyme is either selected from the group consisting of RNase I, RNaseA, RNase4, RNaseT1, RNaseT2, RNase2, and RNase3, or comprises a mixture thereof.

79. The method of claim 77, wherein said enzyme is RNase I.

80. The method of any of claims 77-79, wherein said RNA/DNA hybrid is a product of normalization.

81. The method of any of claims 77-79, wherein said RNA/DNA hybrid is a product of subtraction.

82. The method of any of claims 77-79, wherein said RNA/DNA hybrid is the product of a method comprising the steps of normalization and subtraction in any order or of a method comprising a single normalization/subtraction step.

83. A method of isolating single strand cDNA comprising the steps of treating a hybrid comprising RNA nonspecifically bound to cDNA with an enzyme capable of degrading single strand RNA, removing the degraded single strand RNA, and recovering the cDNA.

84. A method of preparing normalized and/or subtracted cDNA comprising the steps of adding an enzyme capable of degrading single strand RNA driver nonspecifically bound to cDNA tester, and removing the degraded single strand RNA driver.

85. The method of any of claims 77-84, wherein said DNA or cDNA is long-chain, full-coding, and/or full-length cDNA.

Sub
A15

86. The method of any of claims 1-85 employed to prepare one, two, or more libraries.

87. cDNA or a cDNA library obtainable by any of the methods of claims 1-86.

88. The cDNA of claim 87 that is single strand or double strand.

0935592.032401